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EFFECT OF SOMATOSTATIN AND ITS ANALOGUE ON PROLIFERATION OF HUMAN EPIDERMOID CARCINOMA CELLS IN VITRO

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SUMMARY: Somatostatin (SS) and its analogue inhibit the growth of some human tumors in vivo. Surprisingly, we found that SS-14 and its analogue (SMS 201-995) exhibited unexpected proliferative effects on two lines of cells, A431 and KB human epidermoid carcinoma cells, in vitro. The level of intracellular cyclic AMP, an important second messenger that controls the growth of A431 cells, was unaffected in A431 cells by either SS-14 or SMS 201-995. In contrast, SS-14 (20 nM) and SMS 201-995 (10 nM) reduced the level of intracellular inositol 1,4,5-trisphosphate rapidly but transiently.

Somatostatin (SS) and its analogue potently inhibit the secretion of such hormones as growth hormone, glucagon, gastrin, vasoactive intestinal polypeptide and insulin, and provides benefit in treating patients with tumors that produce these hormones (1-3). SS inhibits the growth of some types of neoplastic cells both in vitro and in vivo (4-8). The cell growth in vitro of human pancreas tumor cells (MIA PaCa-2), breast cancer cells (MCF-7) and small cell lung cancer cells (HCI-H69) is inhibited by SS (4-6).

A431 and other human epidermoid carcinoma cells are unique in that they possess an unusually high level of epidermal growth factor (EGF) receptors and respond to EGF with an attenuation of cell proliferation (9). A previous report by Mascardo et al showed that SS inhibited rapid centrosomal separation and cell growth by EGF in cells that were mitogenically responsive to EGF (10). It would be of interest to determine whether SS had

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any effect on the growth of A431 cells that respond to EGF with an attenuation of cell growth. Accordingly, we examined the effects of SS-14 and its octapeptide analogue, SMS 201-995, on the growth of epidermoid carcinoma cells in vitro.

MATERIALS AND METHODS

Materials

SS-14 was obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). [Tyr^{11}]SS was obtained from Bachem Inc (Torrance, CA, U.S.A.). Epidermal growth factor (EGF) was obtained from the Earth Co. (Hyogo, Japan). Fetal calf serum (FCS), penicillin and streptomycin were all obtained from Gibco. Cyclic AMP (cAMP) assay kits were purchased from New England Nuclear (Boston, MA, U.S.A.), inositol 1,4,5-trisphosphate (IP_3) assay kits were from Amersham Corp.(Tokyo, Japan).

Cell culture

Five lines of human epidermoid carcinoma cells, A431, KB, HSC-2, HSC-3 and HSC-4, were provided by the Japan Cancer Research Resources Bank (Tokyo Japan). A431 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FCS (FCS/DMEM) in humidified atmosphere of 5% CO_2 in air at 37°C. KB cells were maintained in Eagle's Minimum Essential Medium (MEM) supplemented with 10 % FCS and 1 % nonessential amino acids. HSC-2, HSC-3, and HSC-4 cells were maintained in FCS/DMEM.

Cell growth

A431 cells were seeded into 12-well plates (Coster, MA, U.S.A.) at a density of 1.5x10000 cells /well. After culture for 2 days in FCS/DMEM, the medium was replaced by serum-free DMEM containing 0.3% BSA (BSA/DMEM) with or without test materials. Cells were detached from the dishes by treatment with trypsin-EDTA 4, 6, 8 or 10 days after initial feeding. The number of cells was counted with a Coulter counter (Coulter Electronics, Inc.).

Binding study for SS

[Tyr^{11}]SS-14 was radioiodinated by a modification of Chloramine T method to a specific activity of 100 mCi/mg. The cells grown to confluence in 24-well plates were washed once with ice-cold BSA/DMEM supplemented with 20 mM 4(2-hydroxyethyl)-piperazine ethanesulfonic acid and then incubated with radioiodinated ligand (approximately 20000 cpm) at 4°C for 24 hours in the presence or absence of unlabeled ligand in a total volume of 0.5 ml. The reaction was terminated by removing the medium. Cells were washed with ice-cold medium and solubilized with 0.5 ml of 1N NaOH. Radioactivity in each aliquot was counted with an automated gamma-counter.

Determination of cAMP and IP_3

Subconfluent A431 cells were washed once and incubated in BSA/DMEM containing SS-14 (20 nM) or SMS 201-995 (10 nM) for 3, 10 and 120 minutes at 37°C. Control cells were incubated in BSA/DMEM without test materials for a same duration. At the end of incubation, the medium was removed and the cells were extracted twice with an ice-cold 10% solution of trichloroacetic acid (TCA). After the supernatant was extracted with 3 volumes of H_2O-saturated diethyl ether, the concentration of cAMP was determined using an RIA kit. After further neutralization by the addition of KOH, the supernatant was assayed for IP_3 using protein binding assay kits.

Statistics

Data are expressed as mean ± SD of three separate experiments using different cell preparations. The data were analyzed statistically by Student's t-test. The difference between groups was considered statistically significant when P value was less than 0.05.

RESULTS

Effects of SS-14 and SMS 201-995 on cell growth

SMS 201-995 stimulated the growth of A431 cells placed in serum-free medium, with the maximal effect seen 4 days after the initial addition of sample (Fig. 1). Both SS-14 and SMS 201-995 dose-dependently stimulated the growth of A431 cells (Fig. 2). Although this effect was detected at a concentration of 10 nM for both peptides, SMS 201-995 was more potent in this respect. Both peptides stimulated the incorporation of tritiated thymidine into the DNA of A431 cells (data not shown). When the effect of SMS 201-995 on cell growth was evaluated in other human epidermoid carcinoma cells, only the KB cells responded to SMS 201-995 with increased growth, while the HSC-2, 3, or 4 cells did not (Fig. 2).

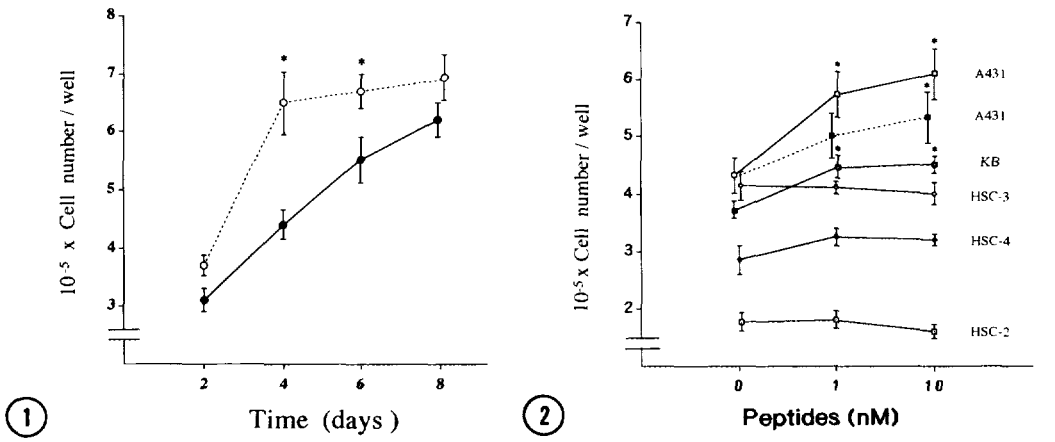


Fig. 1. Time course of growth of A431 cells following treatment with SMS 201-995.
Cells grown in FCS/DMEM were washed with BSA/DMEM and then cultured in BSA/DMEM with (○---○) or without (●—●)SMS 201-995 (100 nM). Media and SMS 201-995 were changed every 2 days. Cells were counted 2, 4, 6 and 8 days after the initial addition of SMS 201-995. Values are mean ± SD (bars) of three determinations.
(*, $p < 0.05$, compared with control)

Fig. 2. Effect of SS on growth of various lines of human epidermoid carcinoma cells.
Cells grown in appropriate media containing 10% FCS were washed with serum-free media and then cultured in the same media with or without test materials (SMS 201-995: —; SS-14: ---). Media and test materials were changed every 2 days. Cells were counted 4 days after the initial addition of test materials. Values are mean ± SD (bars) of three determinations.
(*, $p < 0.05$, compared with control)

Binding study

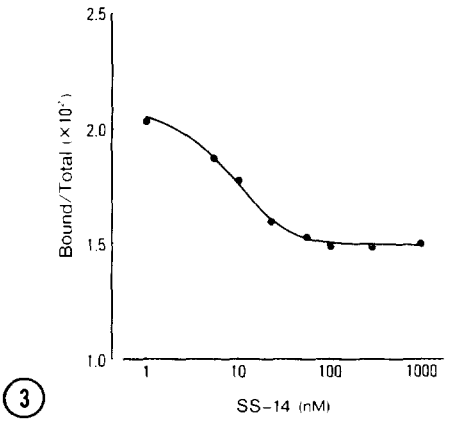
The response of the A431 cells to SS-14 or SMS 201-995 in this study suggested the presence of receptors for SS, confirmed by a study of specific binding of [^{125}I-Thy^{11}]SS-14 to A431 cells. In a pilot experiment, we found that binding was dependent on the duration and tempreture of incubation, so that we subsequently employed the appropriate conditions. Fig. 3 shows that the binding of [^{125}I-TYR^{11}]SS-14 to cells was dose-dependently inhibited by unlabelled SS-14, with the half maximal inhibition observed at 10 nM. Scatchard analysis of the binding data revealed a single class of binding sites with high affinity (Kd = 1.46 nM) and a capacity of 1.14x10000/cell.

Involvement of cAMP

We determined whether cAMP was involved in SS-treated A431 cells. Treating the cells with SS-14 or SMS 201-995 failed to alter intracellular level of cAMP (Fig. 4).

Role of IP_3

Treatment with SS-14 (20 nM) produce a transient decrease the intracellular level of IP_3 (Fig. 4). Level of IP_3 in the cells treated with SS-14 were significantly lower than those



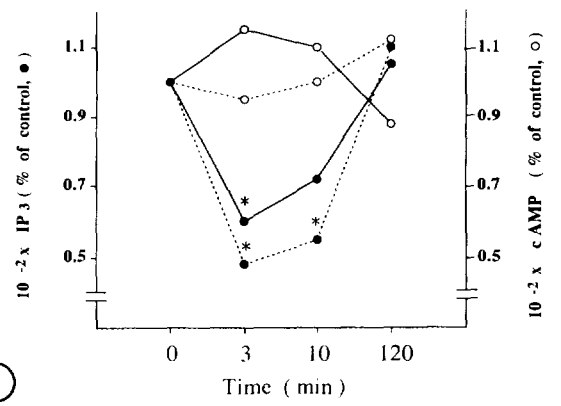


Fig. 3. Displacement of [^{125}I-Tyr]SS-14 by increasing the amount of unlabeled SS-14 using confluent A431 cell culture.
Individual data points represent the average value of duplicate determinations. Scachard analysis of these data demonstrated that the calculated Kd was 1.46 nM and B max was 1.14 x 10^4 /cell.

Fig. 4. Kinetics of cAMP and IP_3 formation by SS.
Monolayers of A431 cells grown in BSA/DMEM were incubated for various periods with SMS 201-995 (10 nM, —) or SS-14 (20 nM, ---). Incubation was terminated by adding TCA. Intracellular cAMP and IP_3 was determined as described in Materials and Methods. Data are expressed as the percentage change, which was defined as 100%. Each value represents the mean of measurements obtained from six monolayers in two different experiments.
(*, $p < 0.05$, compared with control)

in control cells at 3 minutes. The value at 60 minutes did not differ from the control. Similar results were observed with the SMS 201-995-treated cells.

DISCUSSION

We found that the growth of A431 cells in vitro was stimulated by SS-14 as well as by its analogue. We also demonstrated the presence of high-affinity receptors specific for SS. The affinity of receptors was similar to that reported for other tissues (11). The growth stimulatory effect of SS was most likely mediated by an interaction of SS with receptors because the concentrations required to elicit the stimulatory effect was close to the Kd of the receptors. A stimulatory effect was also observed in KB, another lines of epidermoid carcinoma cell, but not in the three lines of squamous cell carcinoma cells, HSC-2, 3, and 4. Although these squamous cell carcinoma cells resemble the A431 and KB cells in that they respond to EGF with a decrease in growth, it appears that the growth regulatory mechanism in the two groups of cells differs with respect to SS. Alternatively, HSC-cells may lack SS receptors.

Recently, Ishizuka et al. reported that SS-14 and its analogue, SMS 201-995, stimulated the growth of pancreas carcinoid cell lines in vitro (7), although they had previously reported an had inhibition of the same cell tumors in vivo (8). In our knowledge, their study is only one to describe a growth stimulatory effect of SS in vitro. In an unpublished study, we also observed that SMS 201-995 seemed to inhibit the growth of A431 cell tumors which were xenotransplanted in athymic Balb/c nude mice. This discrepancy between these in vitro and in vivo findings is of interest.

SS was initially isolated by its ability to inhibit the release of growth hormone from pituitary cells. This hormone appears to inhibit the release of growth hormone, at least in part, by inhibiting the production of cAMP. cAMP-dependent A kinase is involved in growth regulation in a number of cells including A431 cells. A study by Fox et al. showed that an increase in intracellular cAMP levels inhibits the growth of A431 cells (12). In addition, the report by Ishizuka et al. demonstrated that the cell proliferative effect of SMS 201-995 was accompanied by an increased level of intracellular cAMP. However, We found no difference in the intracellular levels of cAMP levels between the SS-treated versus the control A431 cells. Thus, an involvement of cAMP seems unlikely.

Stimulation of cultured cells with a number of growth factors is frequently accompanied by the appearance of intracellular IP_3 derived from phosphatidylinositol

breakdown. In A431 cells, EGF has been shown to stimulate IP_3 production and inhibit the cell growth (13). The decreased level of intracellular IP_3 observed in the SS-treated A431 cells is compatible with the idea that SS stimulates the growth of A431 cells by inhibiting the production of IP_3 production. Additional studies are required to clarify the mechanism by which SS inhibits IP_3 production.

In conclusion, SS-14 and SMS 201-995 stimulated the growth of A431 and KB human epidermoid carcinoma cells in vitro, probably as a result of their effect on inositolphosphatase metabolism.

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